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Research Article

Exploring the therapeutic potential of cinnamon and clove essential oils for mitigating foodborne diseases: bioactivity and molecular interactions

Rithish Jayakumar Pranav¹, Sasidharan Satheesh Kumar^{1✉}, Pugalenth Ragul Rangaraj¹, Karthikeyan Sangapillai¹, Ramachandra Ragunathan²

¹ Department of Biotechnology, Faculty of Engineering, Karpagam Academy of Higher Education (Deemed to be University), Pollachi Main Road, Eachanari Post, Coimbatore, Tamil Nadu, India

² Department of Biotechnology (Bionanotechnology), Centre for Bioscience and Nanoscience Research, Eachanari, Coimbatore, Tamil Nadu, India

Abstract

Foodborne diseases remain a global concern due to their widespread impact on public health. Contaminated food can lead to outbreaks of illness that affect millions of people each year, leading to significant economic burdens and strain on healthcare systems. This study delves into the potential of cinnamon and clove essential oils to combat foodborne diseases. Essential oils extracted via the Soxhlet method using ethanol as solvent, these oils exhibit potent antibacterial, anti-inflammatory, antioxidant and anticancer properties. Gas chromatography-mass spectrometry analysis was performed to analyze the chemical constituents in both essential oils. Employing the Lipinski rule, bioactive compounds are screened and docked against a viral replication responsible protein of norovirus and the neurotoxin (botulinum toxin) of *Clostridium botulinum* both responsible for severe foodborne illnesses. Acetylugenol demonstrates strong binding affinity, while the pyrimidine derivative from cinnamon sourced from Mbeya, Tanzania forms pi-sulfur interactions with both target molecules. This research emphasizes how essential oils confront foodborne diseases, granting valuable perspectives on potential compounds for extended examination and therapeutic interventions.

Keywords

essential oils, foodborne diseases, docked, acetylugenol, pyrimidine derivative and pi-sulfur interactions

Abbreviations

CBEO – cinnamon bark essential oil; CEO – clove essential oil; DMSO – dimethyl sulfoxide; DMEM – Dulbecco's modified eagle medium; DPPH – 2,2-Diphenyl-1-picrylhydrazyl; ELISA – enzyme linked immunosorbent assay; FTIR – fourier-transform infrared spectroscopy; GC-MS – gas chromatography mass spectrometry; HeLa – Henrietta lacks; MHA – Mueller Hinton agar; MSD – mass-selective detection; MTT assay – 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide; OD – optical density; PBS – phosphate buffered saline; RNA – ribonucleic acid; SDF – structure-data file; UV – ultraviolet

✉Corresponding author: Assis. Prof. S. Satheesh Kumar, M.Tech, Department of Biotechnology, Faculty of Engineering, Karpagam Academy of Higher Education (Deemed to be University), Pollachi Main Road, Eachanari Post, Coimbatore - 641021, Tamil Nadu, India, ph.: +918610958770; E-mail: satheesh.sasidharan@kahedu.edu.in

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Introduction

Food spoilage and foodborne diseases are two distinct but interconnected aspects of food safety. Food spoilage can be described as an impairment in food quality, which makes it, unfit for intake. Although it may not always result in sickness, food spoilage can induce gastrointestinal distress and, in some instances, food poisoning. Foodborne diseases occur when pathogenic microbes, parasites, or their toxins contaminate food and consumption of these food products leads to various symptoms ranging from mild discomfort to severe illness. Foodborne diseases impact approximately 600 million individuals globally, leading to a devastating loss of about 420,000 lives (Lee and Yoon 2021). *Salmonella*, *campylobacter*, norovirus (Winder et al. 2022), and *Escherichia coli*, a major pathogen involved in this global concern (Bai et al. 2023). Toxins produced by certain bacteria, such as *Staphylococcus aureus* and *Clostridium botulinum* further contribute to the occurrence of foodborne diseases. The abundance of microorganisms in the surrounding environment means that they can effortlessly contaminate food at various points during harvesting, slaughtering, processing and packing. Chemical preservatives pose potential health risks, contribute to environmental pollution and may lead to drug resistance strains. Thus, novel and eco-friendly methods are required to mitigate the transmission of dangerous infections and improve the longevity of food products without making use of chemical preservatives. Crude extracts made from various medicinal parts of plants, such as the roots, stems, fruits, blossoms, and branches, have been used widely as conventional treatments for a variety of human ailments for a number of years. Due to their established antimicrobial activity against a wide spectrum of bacteria and their perceived therapeutic features, these natural plant extracts have been employed in treatments (Gonelimali et al. 2018). This proved helpful in preventing infections and enhancing health. With these remarkable properties of medicinal plants, we have specifically chosen clove and cinnamon (Ogwaro et al. 2022), as they are not only commonly used in cooking as flavoring agents but also possess potential medicinal benefits. Our research focuses on the extraction of essential oils, comprehensive

analysis of essential oils, as well as early phytochemical studies, notably examining their anti-bacterial, anti-oxidant, and anti-inflammatory capabilities. To validate the presence of active biocompounds, we utilized UV scanning, FTIR analysis and GC-MS. Lastly insilco activity of biocompounds with pharmacological properties present in clove essential oil and cinnamon essential oil evaluated against viral replication responsible protein of norovirus and the neurotoxin of *Clostridium botulinum* respectively.

Materials and Methods

Preparation of plant extracts. *Cinnamomum aromaticum* bark was collected from Mbeya city, Southwest Tanzania, Africa and *Syzygium aromaticum* bud was purchased from a local vendor, in Coimbatore, Tamil Nadu, India. Bark and clove were subjected to a thorough cleansing with distilled water and left to air dry for a duration of 48 h. After drying, bark and clove were ground into powder and packed with crude filter paper to proceed with Soxhlet extraction. For 100g cinnamon bark and clove sample, 170mL and 150mL of absolute ethanol were used for extraction at 60°C. Soxhlet ran until the colour changed in the solvent. Later the ethanolic crude extract was collected and proceeded with a rotary evaporator (40-60°C) in order to remove the ethanolic residues and then essential oils of cinnamon bark and clove were obtained (Wong et al. 2014; Janani et al. 2019).

Antibacterial activity. The antibacterial potency of essential oils was tested against the following food-associated pathogens; *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus anthracis*, *Pseudomonas aeruginosa* and *Escherichia coli*. MHA agar plates were prepared and 150µL of each bacterial strain were inoculated and spread at their respective plates using a sterile L rod. A pinch of crude essential oil extract was taken and resuspended into DMSO to perform Agar well diffusion method (Chavez-Esquivel et al. 2021). Wells were prepared using sterile cork borer and 100µL of CEO, CBEO, the combination of CEO and CBEO (1:1) and DMSO were added into their respective wells. DMSO serves as a negative control (Abdelhamed et al. 2022).

Anti-inflammatory activity. The albumin denaturation method (Matulja et al. 2021) was performed to analyse the anti-inflammatory property of plant extracts. 10mg.mL⁻¹ of both essential oil extracts were taken in two different test tubes and ethanol was used as a solvent for suspending the crude to obtain the concentration. To the tubes, 3 mL of PBS and 1mL of egg albumin are added and mixed and incubated at 37°C for 20min. Denaturation was induced by increasing the temperature to 90°C (Gowtham and Pranav 2022) for 2-3 min. Absorbance was taken at 660nm after cooling down the mixture. As a positive control, diclofenac was utilized, while distilled water serves as the negative control. The percentage of denaturation inhibition is calculated by Equation 1:

$$\text{Inhibition(\%)} = \frac{\text{absorbance of control} - \text{absorbance of test}}{\text{absorbance of control}} \times 100 \quad (1)$$

Antioxidant activity. DPPH assay was performed by modifying the protocol of Lin et al. (2016) to examine the Anti-oxidant properties of cinnamon and Clove Eos. Both extracts were taken at five different concentrations 10, 25, 50, 75 and 100 µg.mL⁻¹. Concentration was obtained by diluting the crude essential oil extract using ethanol. 0.5 mL of respective essential oils and their concentration were taken in separate tubes. To each tube, 0.2mL of 0.1mM DPPH is added and mixed. Then incubated for 5 min. After the incubation, 0.4mL of 50mM TrisHCl was added and following a period of around 30 min in a dark environment, the absorbance was measured at 517nm (Aboukhalaf et al. 2023). The percentage of inhibition was determined using the formula typically employed for assessing anti-inflammatory properties. Ascorbic acid served as positive control. Combination of ethanol and DPPH as the negative control.

Anticancer activity. The HeLa cell line cultures were cultivated in DMEM medium under controlled conditions within a CO₂ incubator, maintaining 5% CO₂, 80% humidity, and a temperature of 37°C. After a 48 h cultivation period, cellular viability was assessed through microscopic examination using an inverted microscope, followed by analysis with a microplate reader (Johneey et al. 2018). For the investigation, distinct concentrations of cinnamon and clove essential oil samples were meticulously prepared, encompassing a concentration range of 5

to 25 µL. Each prepared sample, in conjunction with the HeLa cell line, was introduced, and a control group comprising solely the cell line was concurrently established. The cellular entities were subjected to a 24 h incubation period. Subsequent to the incubation phase, cells underwent sequential treatment involving DMSO and trypsin. Following this, 20 µL of the MTT dye was introduced to each well. After an additional 24 h, optical density (OD) measurements were taken at a wavelength of 570 nm employing an ELISA reader (Robonik). This comprehensive procedural framework offers insights into the potential cytotoxic effects of varying concentrations of cinnamon and clove essential oils on the HeLa cell line, encompassing the specific incubation and treatment sequences.

Phytochemical analysis. Preliminary phytochemical screening was performed on CBEO and CEO according to Adarsh et al. (2020).

UV scan and FTIR. UV-visible spectrophotometric analysis was performed on cinnamon bark and clove Eos, utilizing a UV-visible spectrophotometer (Labtronics LT-297). The crude essential oils of cinnamon bark and clove were rediluted into absolute ethanol at a 1:10 ratio prior to the analysis and wavelength range parameters were set between 200-800nm for both. The translucent discs of CBEO and CEO extracts were prepared using KBr. Discs were loaded in FTIR spectroscope with scan range from 500-4000 cm⁻¹ (Shimadzu).

GC-MS analysis. The crude essential oils of cinnamon bark and clove were diluted using DMSO and centrifuged in order to remove debris. The supernatants were collected and GC-MS analysis was performed. GC-MS (Gas chromatography mass spectrometry) analysis was conducted using Agilent technologies 7820A GC system with a DB-5 column (30m length, 0.25mm internal diameter, 0.25µm film thickness). To enable mass spectral detection, the GC system was connected to the MSD through a suitable interface. Temperature was initially set at 100°C and programmed to increase at a rate of 10°C per min until reaching a final temperature of 270°C. Helium gas was employed as the carrier gas, flowing at a rate of 1.2 mL.min⁻¹. The chromatographic data and mass spectra were acquired and processed using mass hunter software (Agilent Technologies).

Molecular docking. Chemical compounds obtained after GC-MS analysis were screened based on Lipinski rule in order to select potential candidates. Two specific targets were selected for the docking study: the RNA-dependent RNA polymerase of norovirus (PDB ID: 4LQ9) and Botulinum neurotoxin A3 Hc domain (PDB ID: 6F0o). From the PubChem database, we retrieved the 3D structural information of chosen chemical compounds in the SDF format, while the corresponding protein structures were derived from the Protein Data Bank. Using the chimera software (Pettersen et al. 2004) the molecules were prepared for subsequent analysis. Molecular docking simulations were then carried out using autodock vina software (Trott and Olson 2010) via chimera software along with specific grid parameters set to each protein molecule (for 4LQ9, center X=-24.33, Y=-27.75, Z=-11.61 and size X=39.45, Y=49.94, Z=42.20 and for 6F0o, center X = 8.33, Y=-2.45, Z=14.90 and size X=28.51, Y=25.39, Z=23.75). The resulting interactions of each compound evaluated in terms of binding energy (kcal.mol^{-1}) binding pose with the least binding energy were chosen for all compounds. To visualize and comprehend the interactions, the chosen compound and their respective targets were analysed using biovia discovery studio software.

Results and Discussion

Antibacterial activity. The study found that both CBEO and CEO exhibited antibacterial activity against all the organisms they were tested on. However, the zone of inhibition varied depending on the specific bacterium being examined (Table 1). In the results, it was observed that cinnamon oil exhibited potent activity against Gram-negative bacteria, while clove oil demonstrated effectiveness against Gram-positive bacteria. Notably, both cinnamon and clove oil displayed activity against drug-resistant *E.coli* strain, but the combined effect of both oils shows a minimal effect compared to their individual potency. DMSO was added to the control well on each plate for respective organisms, and no antibacterial activity was observed, resulting in the absence of a zone of inhibition.

Anti-inflammatory activity. The effectiveness of the plant extracts that are anti-inflammatory in nature has been assessed using the albumin denaturation method. Essential oil extracts of both cinnamon and clove were tested at 10 mg.mL^{-1} concentration, utilizing this technique.

Table 1. Zone of inhibition (diameter) of CBEO and CEO against tested bacteria

Bacteria employed and their gram stain	CBEO, cm	CEO, cm	CBEO+CEO, cm	DMSO, cm
<i>Staphylococcus aureus</i> (+ve)	2.1	2.6	1.5	-
<i>Salmonella typhi</i> (-ve)	2.5	2.1	1.4	-
<i>Bacillus anthracis</i> (+ve)	1.7	2	1.1	-
<i>Pseudomonas aeruginosa</i> (-ve)	2.9	2.1	1.6	-
<i>Escherichia coli</i> (superbug) (-ve)	1.8	1.9	1.0	-

The outcomes exhibited significant inhibitory effects on albumin denaturation, with cinnamon oil showcasing a notable inhibition rate of 63.22%, and clove oil demonstrating an inhibition rate of 62.86%. These findings underscore the promising anti-inflammatory attributes inherent in both cinnamon and clove essential oils, thereby designating them as prospective entities for further investigation as natural anti-inflammatory agents. In

the study by Manaswini et al. (2019), a concentration of $100\mu\text{g.mL}^{-1}$ of clove oil inhibited egg albumin denaturation by 80%, and likewise cinnamon oil at a lower concentration exhibits a maximum inhibition of denaturation (Praveen et al. 2018). Notably, for both oils, lower dosages were more potent in inhibiting protein denaturation than higher dosages.

Antioxidant activity. The DPPH radical assay represents a suitable model to evaluate the total antioxidant potential of cinnamon bark and clove essential oils. The results represented in a graph shown in Fig. 1, demonstrated a clear trend, increasing the concentration of both cinnamon and clove essential oils resulted in a corresponding increase in inhibitory percentage. Notably, both essential oils at 100 $\mu\text{g.mL}^{-1}$ concentration inhibited 94.8% (CBEO) and 97.1% (CEO) that exhibited

superior antioxidant activity compared to ascorbic acid (93.8% at 100 $\mu\text{g.mL}^{-1}$). However, the graph revealed that clove essential oil showed a steeper and more pronounced increase in inhibitory percentage with increasing concentration, indicating its higher potency. At 200 $\mu\text{g.mL}^{-1}$ concentration, clove oil demonstrated complete antioxidant efficacy of 100%, as indicated in Ghadermazi et al. (2017).

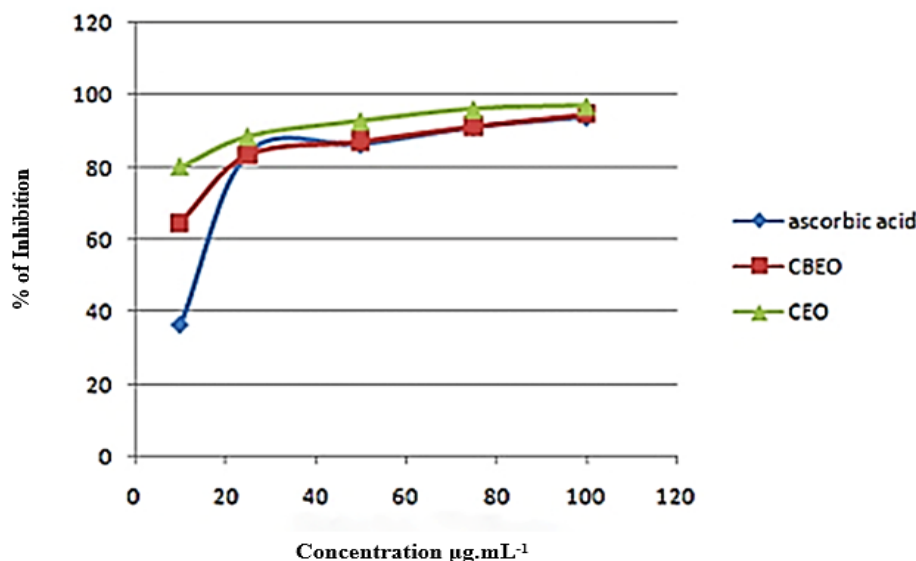


Figure 1. DPPH inhibition graph

Anticancer activity. The investigation involved evaluating the cytotoxic effects of cinnamon and clove essential oils on the HeLa cell line, employing concentrations ranging from 5 to 25 μL . The resulting data is summarized in the Table 2. In comparison, the control group, consisting solely of the HeLa cell line, maintained a consistent viability of 100%. This showcased the promising potential of both cinnamon and clove essential oils in influencing cell viability. As concentrations varied from 5 to 25 μL , both essential oils displayed concentration-dependent trends in reducing cell viability. At 5 μL , both cinnamon and clove essential oils exhibited considerable cytotoxic activity, with cell viabilities of 82.14% and 86.74%. The intensification of cytotoxic effects was evident at 25 μL , as cinnamon oil led to a cell viability of 51.21%, and clove oil yielded a viability of 60.79%. These findings highlight a concentration-dependent reduction in cell viability for both essential oils,

underscoring their potential as agents for restraining the proliferation of cancer cells (Iwiński et al. 2023).

Table 2. Effects of cinnamon bark essential oil (CBEO) and clove essential oil (CEO) on HeLa cell viability

Concentration of sample used, μL	% of cell viability	
	CBEO	CEO
5	82.14	86.74
10	72.38	75.29
15	64.94	70.66
20	58.36	64.13
25	51.21	60.79
Control	100	100

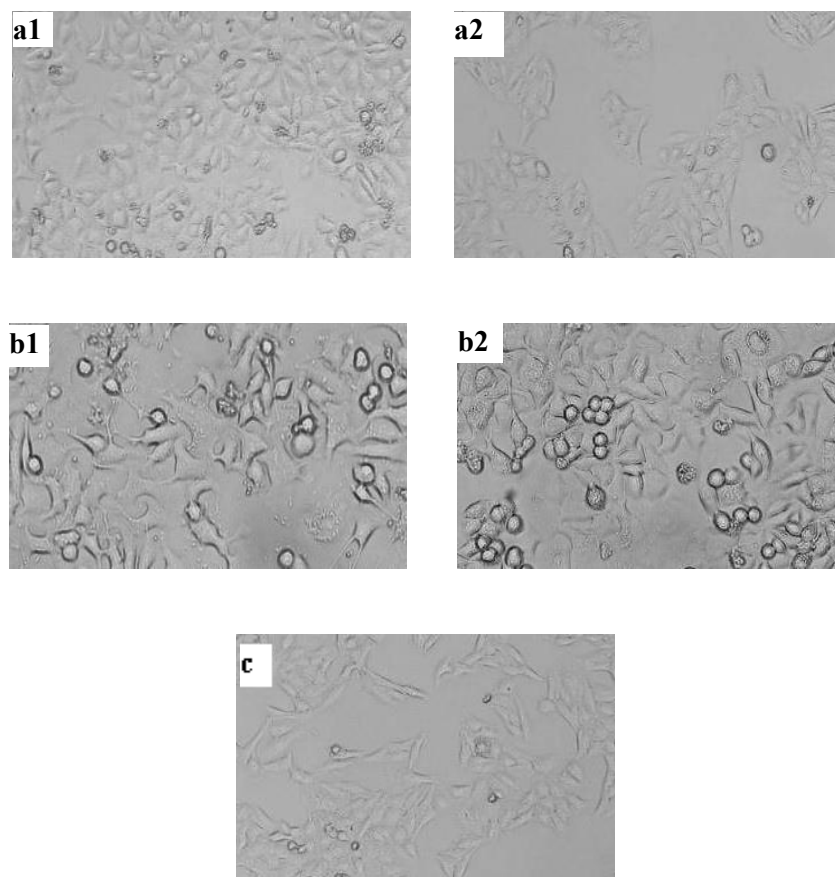


Figure 2. Microscopic image of HeLa cells treated with CBEO (a1-5 μ L, a2-25 μ L) and CEO (b1-5 μ L, b2-25 μ L). C- Control

Phytochemical constituents of CBEO and CEO

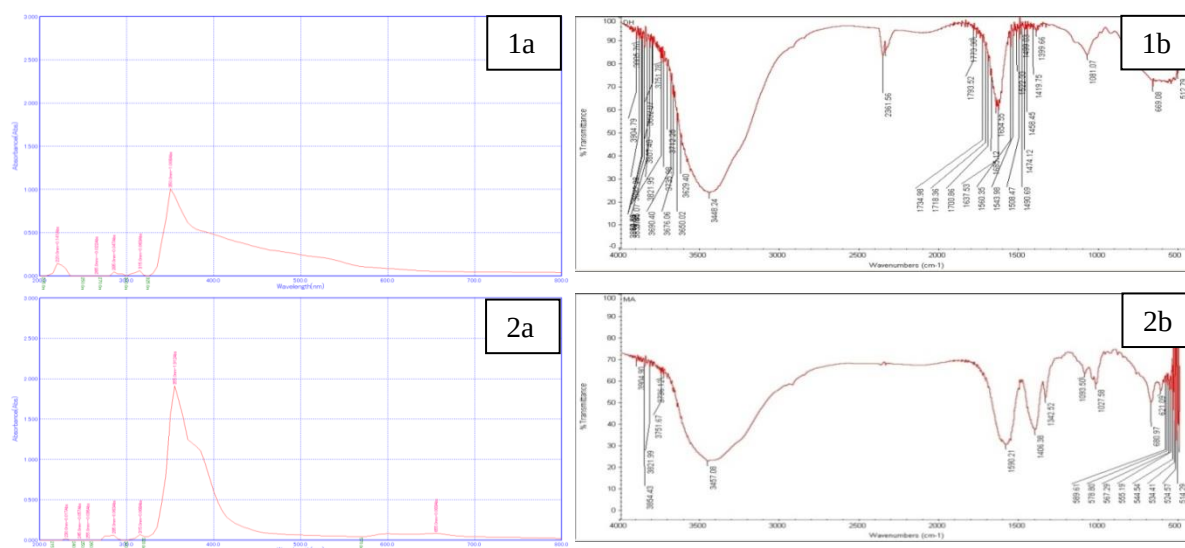


Figure 3. UV and FTIR spectral data of CBEO (1a, 1b) and CEO (2a, 2b)

Alkaloids, polysaccharides, phenols, steroids, and terpenoids were all clearly observed in the initial phytochemical screening of both CBEO and CEO. Spooning were detected in CBEO, which highlights its distinctive composition. Conversely, flavonoids, proteins, coumarins, and quinines were absent in both extracts. Both UV scan and FTIR (Hemalakshmi et al. 2017) spectral data of CBEO and CEO shown in Fig. 3 indicates the presence of phytocompounds; the FTIR spectral peaks with their corresponding wavenumbers (cm^{-1}) in Fig. 3 (1b and 2b) demonstrates the functional groups such as hydroxyl, alcohol, alkyne, anhydride, aldehyde, alkene, nitro compound, carboxylic acid, thioketone, halo compounds, amine, polysaccharides, and aromatic amines (Siejak et al. 2021) in the essential oils (Hari and Nair 2018).

The results presented by the GC-MS chromatograms (Fig. 4) indicated the presence of bioactive compounds within CBEO and CEO. The major bioactive compounds present in CBEO are eugenol (2.21%), methyl dimethoxysilane (4.97%), 6-amino-2-(methylthio) pyrimidine-4-ol (76.81%), tridecanoic acid (2.05%), oleic acid (3.40%), cis 6-octadecenoic acid (3.25%), and octadecanoic acid (4.97%). Likewise in CEO, the major compounds are eugenol (78.28%), cis-caryophyllene (2.90%), caryophyllene (4.25%) and acetyl eugenol (14.57%). The pyrimidine derivative was reported in CBEO which can be synthesized from other chemical constituents in Alwan et al. (2023) the possible chemical reactions to synthesize heterocyclic constituents from camphor were illustrated.

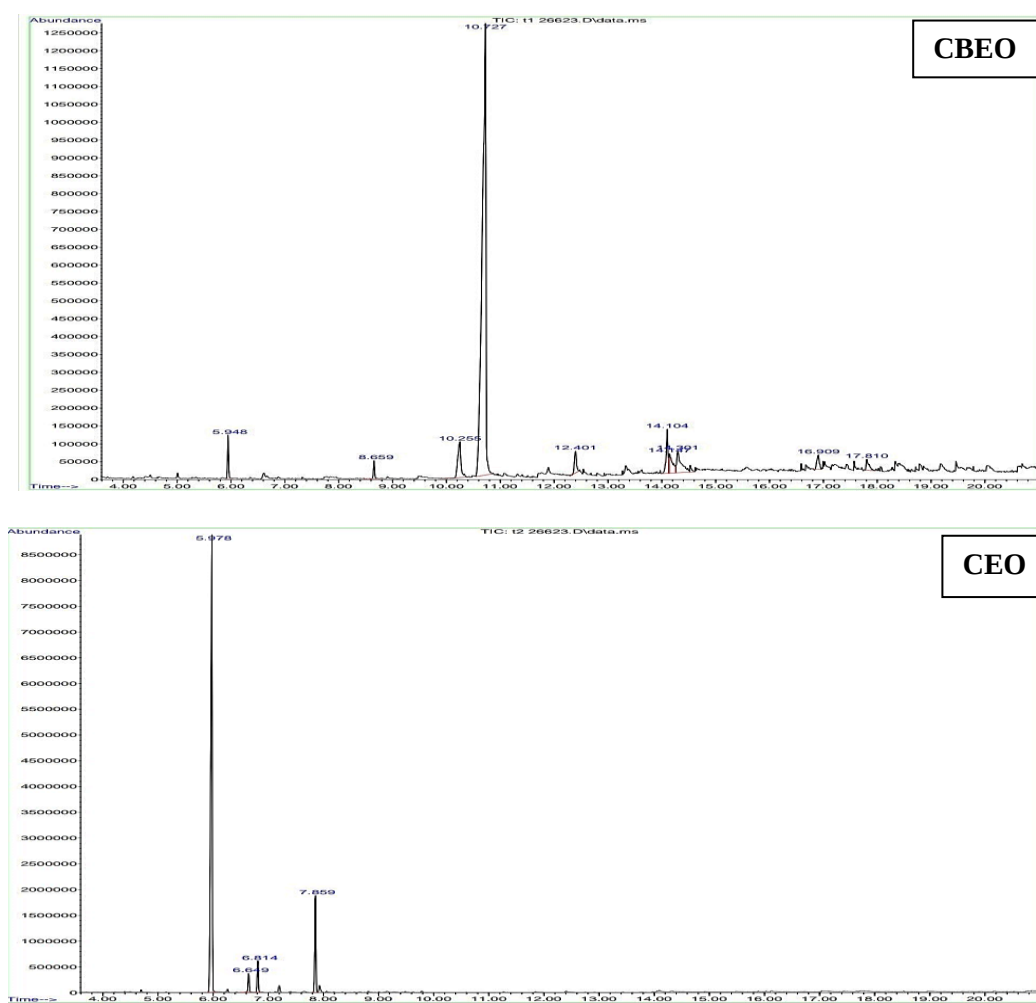


Figure 4.GC-MS chromatogram of CBEO and CEO

Molecular docking. Eugenol, acetyl eugenol and 6-amino-2-(methylthio) pyrimidine-4-ol were the phytochemicals from GC-MS results that were taken for molecular docking by screening on the basis of the Lipinski rule (Gowtham and Pranav 2024). These compounds were docked against the

RNA-dependent RNA polymerase of norovirus and Botulinum neurotoxin A3 Hc domain. The docking score for norovirus protein for eugenol is -5.8 kcal.mol⁻¹, acetyl eugenol has -6.0 kcal.mol⁻¹ and 6-amino-2-(methylthio) pyrimidine-4-ol has -5.2 kcal.mol⁻¹.

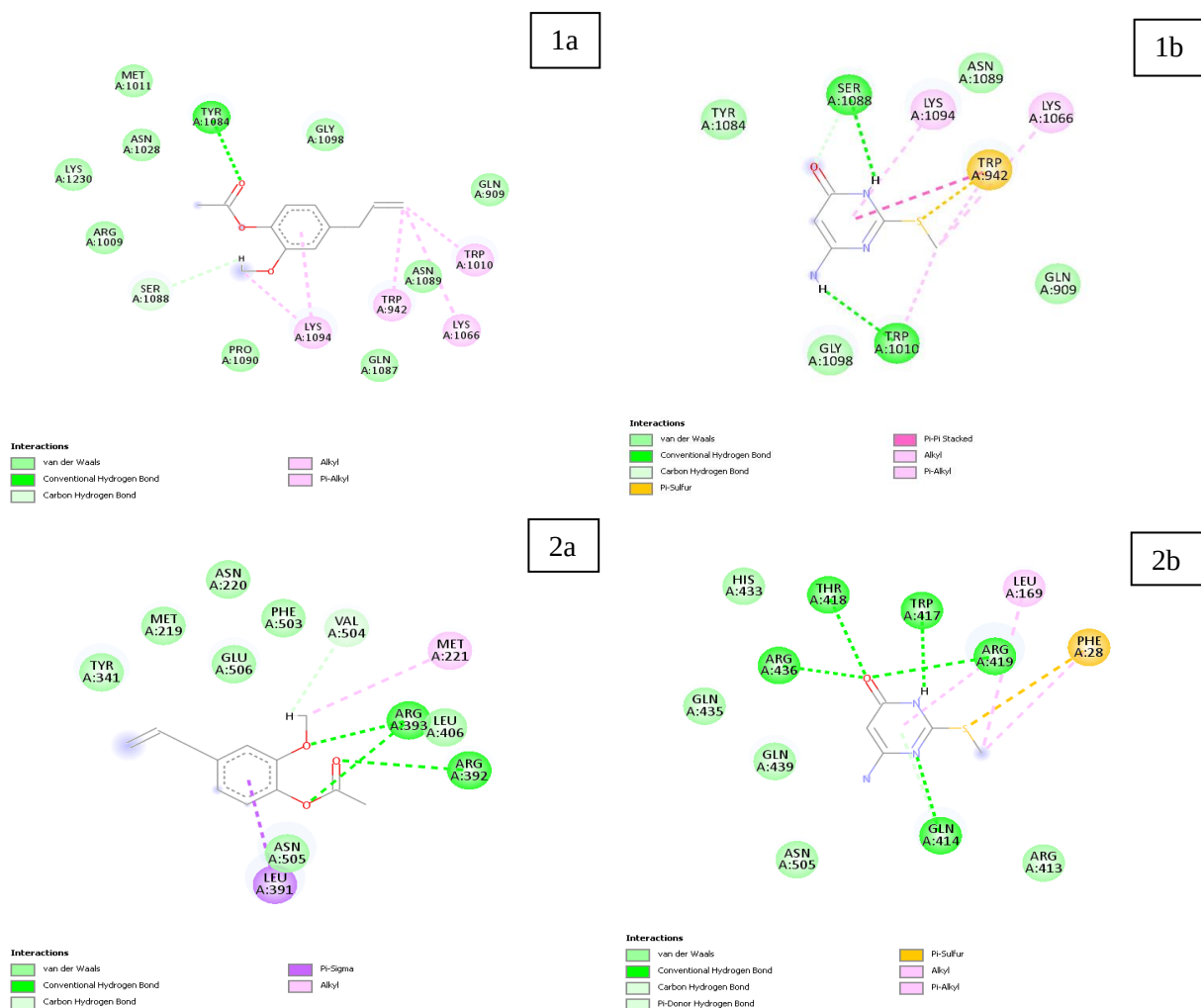


Figure 5.2-D representation of protein-ligand interactions.

(botulinum neurotoxin (1a) acetyl eugenol & (1b) Pyrimidine, 2-methylthio-4-hydroxy-6-amino. Norovirus (2a) acetyl eugenol & (2b) Pyrimidine, 2-methylthio-4-hydroxy-6-amino.

Against bontoxilysin, eugenol has -5.9 kcal.mol⁻¹, acetyeugenol has -6.5 kcal.mol⁻¹ and 6-amino-2-(methylthio) pyrimidin-4-ol has -4.8 kcal.mol⁻¹. The docking analyses conducted against bontoxilysin and norovirus proteins (Fig. 5) reveal intriguing insights into the interactions between bioactive

compounds and these target proteins. Against botulinum neurotoxin, eugenol demonstrates a carbon-hydrogen bond with SER A: 1088 and engages in pi-alkyl, alkyl, and pi-pi stacked interactions with LYS A: 1066, TRP A: 1010, TRP A: 942, and LYS A: 1094. Vander Waals

interactions stabilize the binding involving ASP A: 906, GLN A: 909, TYR A: 1084, ASN A: 1089, SER A: 1088, GLN A: 1087, and PRO A: 1090 residues. Acetyl eugenol forms a single conventional hydrogen bond with TYR A: 1084, a carbon-hydrogen bond with SER A: 1088, and alkyl and pi-alkyl with bondings LYS A: 1094, TRP A: 942, LYS A: 1066 and TRP A: 1010. Vander Waals interactions are observed with LYS A: 1230, ARG A: 1009, ASN A: 1028, MET A: 1011, GLY A: 1098, GLN A: 909, ASN A: 1089, GLN A: 1087, and PRO A: 1090 residues. 6-amino-2-(methylthio) pyrimidine-4-ol engages in molecular interactions through the formation of two hydrogen bonds with SER A: 1088 and TRP A: 1010, as well as carbon-hydrogen bond with SER A: 1088. Pi-sulfur and pi-pi stacked interactions are observed with TRP A: 942. Alkyl and pi-alkyl interactions take place with LYS A: 1094 and LYS A: 1066, and Vander Waals interactions occur with TYR A: 1084, ASN A: 1089, GLN A: 909, and GLY A: 1098 residues. Shifting the focus to the norovirus protein, the docking results illuminate the interactions of these compounds in the context of antiviral applications. Eugenol's docking showcases a conventional hydrogen bond and unfavorable donor-donor interaction with ARG A: 393 and intriguing carbon-hydrogen bond interactions with LEU A: 391. Alkyl and pi-alkyl bonds with TYR A: 341, LEU A: 391, and LEU A: 406 further cement its presence. These interactions are complemented by Vander Waals interactions that extend their engagement to amino acid residues ASN A: 220, ASN A: 505, ARG A: 392, VAL A: 504, MET A: 221, PHE A: 503 and GLU A: 506. On the other hand, acetyl eugenol establishes three conventional hydrogen bonds with ARG A: 392 and ARG A: 393, while also forming a pi-sigma bond with LEU A: 391, additionally, alkyl interactions occur with MET A: 221 and a carbon-hydrogen bond is observed with VAL A: 504. The binding is further influenced by Vander Waals interactions, encompassing residues such as ASN A: 505, TYR A: 341, MET A: 219, GLU A: 506, ASN A: 220 and PHE A: 503. Similarly 6-amino-2-(methylthio) pyrimidin-4-ol forges five conventional hydrogen bonds with ARG A: 419, ARG A: 436, THR A: 418, TRP A: 417, and GLN A: 414. Alongside a pi-sulfur bond with PHE A: 28. Alkyl and pi-alkyl interactions are established with PHE A: 28, LEU A: 169, and ARG A: 419. Vander Waals interactions play a significant role in

engaging, HIS A: 433, GLN A: 435, GLN A: 439, ASN A: 505, and ARG A: 413. These interaction profiles of chemical compounds solidify its engagement within the protein's binding pocket. The aromatic interactions play an essential role in docking for drug development. By enhancing ligand-receptor stability and improving binding specificity through distinct residue interactions, these interactions contribute to the optimization of drug candidates. Facilitating structural orientation and optimizing molecular conformations (Daeffler et al. 2012), they guide the rational design of molecules with enhanced binding affinities. In drug development, these interactions are pivotal for predicting and evaluating potential binding interactions, aiding the selection and optimization of lead molecules (Brylinski2018).

Conclusion

This study unveils the diverse bioactivities of cinnamon bark essential oil (CBEO) and clove essential oil (CEO) extracted via Soxhlet method, uncovering its many health advantages, including its potential to combat cancer, decrease inflammation, serve as an antioxidant, and fight against bacteria. Gas chromatography-mass spectrometry (GC-MS) exposes intricate compositions, notably eugenol enrichment in CEO and cinnamon with geographical authenticity in Mbeya, Tanzania, shows a dominant pyrimidine derivate named 6-amino-2-(methylthio) pyrimidine-4-ol in CBEO. Insilico molecular docking highlights acetyl eugenol's binding affinity efficacy against norovirus viral replication protein and Botulinum neurotoxin of *Clostridium botulinum*, while the unique pi-sulfur interactions of pyrimidine highlight the interaction mechanisms. This research, spanning pharmaceuticals, functional foods, antimicrobials, and cancer therapeutics, holds promise for combating foodborne diseases. Bioengineering and environmental health applications extend their relevance. Integrating experimental data, simulations, and geographical context offers a holistic view of CBEO and CEO's bioactive potential, laying a foundation for innovative therapeutic pathways and emphasizing the role of natural resources in addressing food-related health challenges, alongside potential contributions to advancing biomedicine and molecular design for a safer food supply.

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